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(54) Title: RECOMBINANT LIBRARY SCREENING METHODS

(57) Abstract

Nucleotide sequences encoding proteins of interest are isolated from DNA libraries using bacteriophage to link the protein to the sequence which encodes it. DNA libraries are prepared from cells encoding the protein of interest and inserted into or adjacent to a coat protein of a bacteriophage vector, or into a sequence encoding a protein which may be linked by means of a ligand to a phage coat protein. By employing affinity purification techniques the phage particles containing sequences encoding the desired protein may be selected and the desired nucleotide sequences obtained therefrom. Thus, for example, novel proteins such as monoclonal antibodies may be produced and conventional hybridoma technology avoided.

"RF" DNA of filamentous phage), and to clone the separate libraries in different versions of the vector containing different cloning sites and different selectable markers (Amp^R and Tet^R, for example). The sequences from the separate libraries are joined so that a sequence of interest and a corresponding selectable marker from each library resides in each single phage genome. Application of double selection will enrich for those hosts harboring the combined phage genomes. Alternatively, in accordance with the present invention antibody light and heavy chains can be expressed from separate plasmids.

In other embodiments the V region domains of heavy and light chains can be expressed on the same polypeptide, joined by a flexible linker to form a single-chain Fv fragment, which gene sequences can then be cloned into the desired vector. As generally described in McCafferty et al., Nature 348:552-554 (Dec. 6, 1990), complete antibody V domains of an anti-lysozyme antibody, joined by a flexible $(Gly_4\text{-}Ser)_3$ linker and cloned into an fd phage vector fdCAT1 at the N-terminal region of the gene III protein, produce an antibody-gene III fusion which is detectable in the recombinant phage. The recombinant phage can be affinity-purified over a lysozyme-Sepharose® column and subsequently further enriched by propagating the isolated phage.

In yet another approach, one or both of the antibody light and heavy chains (or the V regions thereof) can be expressed by a phagemid, that is, a vector which combines features of plasmids, such as a bacterial ColE1 origin of replication, and filamentous bacteriophages, such as the major intergenic region. Phagemids are discussed genrally in Sambrooke et al., supra, at pages 4.17-4.19 and 4.44-4.50, which are incorporated herein by reference. The vector can be maintained as a small plasmid vector by selection, or the plasmid can be efficiently packaged into virions, or phagemid particles, by infection with helper phage, such

as M13KO7. As described generally in Bass et al., Proteins 8:309-314 (1990), controlling the copy number of gene III fusion proteins which are produced in a mixture of both wild-type gene III and a gene III fusion protein on phagemid particles permits proper folding of a fusion protein while allowing efficient panning of large epitope libraries. Thus, it is possible to sort phagemid particles whose peptides are of relatively high affinity, with binding constants in the micromolar to nanomolar range.

As mentioned, the vectors may be engineered to efficiently clone the library amplification products. For example, oligonucleotides may be used to introduce the asymmetric restriction sites, a ribosome binding site at an optimal distance for expression of the cloned sequence, and cloning sites for the library amplification products.

In certain embodiments of the invention at least one of the library-encoded protein chains is cloned into a vector so as to be expressed fused to a tag protein. As used herein, a "tag" protein is meant to refer to a protein which has a specific binding affinity for a peptide or protein ligand. For example, the tag protein should have specificity for a peptide or protein ligand of at least about 3 to about 100 or more amino acids, preferably with an affinity exceeding at least about 10^{-7} to 10^{-8}M, and more preferably equal to or greater than about 10^{-9}M. A tag protein with more than one binding site for the tag ligand peptide provides a resulting increase in avidity, which greatly decreases the dissociation rate and somewhat relaxes the requirement for affinity at each site (the affinity of each binding site may then be as low as about 10^{-6}M). The tag protein should be preferably less than about 100kD but may be more, and should be of a composition such that its fusion with the protein of interest, e.g., the N-terminus of the tag with the C-terminus of an